THE INTERFERENCE PHENOMENON IN ALLERGIC CONTACT DERMATITIS*

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Recently we observed that the simultaneous application of two contact allergens of unequal sensitizing capacity did not result in the expected incidences of sensitization; the weaker allergen was blocked. We have called this the interference phenomenon. This paper describes tentatively some of its characteristics.

MATERIALS AND METHODS

The original observations were made with two highly potent contact allergens, 2,4-dinitrochlorobenzene (DNCB) and para-nitrosodimethyl aniline (NDMA). The frequency of sensitization to varying concentrations of each of these was worked out over a period of several years in healthy adult negro males. To sensitize, the agents were dissolved in acetone and 0.25 ml applied within a cup 2.9 cm in diameter under a stream of air (open patch test). After evaporation of the acetone the site was covered with a plastic Band-Aid‡. A similar application at a new site, using a standard concentration of 1:000, was made one month later to determine if sensitization had occurred. The challenge patch tests were observed after 2, 4 and 6 days. The incidence of sensitization for each concentration was as follows:

Concentration	Incidence of Sensitization	
	DNCB	NDMA
0.0005 molar..	3/64 = 5%	0/24 = 0
0.0025 molar..	6/28 = 16%	—
0.005 0.01 molar.....	65/105 = 62%	62/137 = 45%
0.05 molar....	21/23 = 91%	20/25 = 80%
0.5 molar.....	107/120 = 89%	42/53 = 79%
1.0 molar.....	39/45 = 87%	32/45 = 71%

It is apparent that the sensitization-frequency curve is roughly linear, the incidence of sensitization increasing proportionately with the concentration. At equal concentrations DNCB sensitizes a higher percentage of individuals and is adjudged the stronger sensitizer of the two. The sensitization rates were within the expected range when the allergens were applied simultaneously to separate sites, so long as the concentrations were alike or nearly so. We came across the interference phenomenon when it was observed that the simultaneous application of unequal concentrations of these two allergens to separate sites gave frequency values for the weaker one which were decidedly less than expected.

EXPERIMENTAL STUDY

Simultaneous Application of Unequal Concentration of DNCB and NDMA

DNCB, the stronger allergen, was applied in a concentration 100 times that of NDMA. 0.5 molar DNCB and 0.005 molar NDMA were applied simultaneously at different sites in 23 subjects. The resultant frequencies of sensitization were:

	Expected Incidence of Sensitization	Actual Incidence of Sensitization	P Value*
DNCB (0.5 M).....	89%	18/23 = 78%	.18
NDMA (0.005 M)..	45%	3/23 = 14%	<.01

* 0.05 was considered the level of significance.

Whereas, the frequency of sensitization to DNCB fell within the expected range, there was interference with sensitization to NDMA; the incidence was reduced to about one third of the expected value.

The converse experiment in which 0.5 molar NDMA was simultaneously applied with a hundred fold weaker concentration of DNCB (0.005 M) gave the following results in 15 subjects:

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‡ Johnson & Johnson.

	Expected Incidence of Sensitization	Actual Incidence of Sensitization
DNCB (0.005 M)...	62%	9/15 = 60%
NDMA (0.5 M).....	79%	11/15 = 73%

Under these conditions NDMA did not interfere with sensitization to DNCB. At first this was attributed to the fact that 0.005 M DNCB sensitizes almost as many as 0.5 M NDMA and therefore the sensitizing capacities of these two concentrations were not really dissimilar.

The final experiment ruled out this explanation. 15 *white* men were exposed simultaneously to 0.5 molar NDMA and 0.0005 molar DNCB, a thousand fold difference. Suitable control subjects were also tested.

	Expected Incidence of Sensitization (Controls)	Actual Incidence of Sensitization
DNCB (0.0005 M)..	39/131 = 30%	7/15 = 40%
NDMA (0.5 M).....	29/32 = 94%	13/15 = 87%

NDMA still did not interfere with sensitization to DNCB. Apparently, the stronger sensitizer cannot be blocked by the weaker one, no matter what the difference in concentrations may be.

Application of Unequal Concentrations of DNCB and NDMA at Different Times

0.005 molar NDMA was applied 5, 10, 15 and 30 days after an interfering dose of 0.5 molar DNCB. The results were as follows:

Days after 0.5 M DNCB Application	Incidence of Sensitization to NDMA		
	Expected	Actual	P Value
5	46%	3/13 = 15%	.0184
10	46%	1/10 = 10%	.0463
15	46%	2/10 = 20%	>.1
30	46%	5/15 = 33%	>.2

There was definite interference with NDMA sensitization at 5 and 10 days. After 15 days the interference effect was weaker and by 30 days was only questionably present. It may be mentioned that in each case the frequency values for DNCB were in the expected range, namely 100, 80, 90 and 93%.

Application of DNCB and Monobenzyl Ether of Hydroquinone (MEH)

Monobenzyl ether of hydroquinone (MEH) is a weaker contact allergen than either DNCB or NDMA. A single application of a 1 M solution in acetone sensitizes only about 6% of negro males. By applying 0.5 M MEH in an ointment base to skin freshly irritated by freezing (3 second exposure to Freon 12) sensitization can be increased to about 25%. This was done 5 days after an interfering dose of 0.5 M DNCB with the following result:

	Expected Incidence of Sensitization	Actual Incidence of Sensitization	P Value
MEH (0.5 M)..	17/73 = 23%	2/25 = 8%	0.085
DNCB (0.5 M).	89%	23/25 = 92%	

Although statistical significance was borderline due to the small sample, there appeared to be definite evidence of interference, the ratio of the actual to the expected frequency being about 1:3.

Application of NDMA and Less Potent Allergens

Up to this point all the successful experiments had utilized DNCB as the blocking allergen. It was necessary to exclude the possibility that DNCB blocked sensitization by some pharmacologic property peculiar to itself. 0.5 Molar NDMA was utilized as a blocking allergen against formalin, a considerably weaker sensitizer. Both allergens were applied simultaneously. A small paper disc soaked in 2.0% aqueous solution of formalin was applied to a site freshly irritated by freezing and immediately occluded under an impermeable dressing. A control group was similarly treated except that no NDMA was applied. The results were as follows:

	Expected Incidence of Sensitization	Actual Incidence of Sensitization	P Value
2% Formalin.....	26/97 = 27%	2/30 = 7%	0.014

While the incidence of sensitization to NDMA fell within expected limits, that to formalin was evidently depressed. The experiment was repeated. 15 subjects were simultaneously exposed to 0.5 molar NDMA and a full strength extract of krameria, another weak sensitizer. The method

of application of krameria was the same as for formalin. At the same time control subjects were tested with krameria alone. The results were as follows:

	Expected Incidence of Sensitization	Actual Incidence of Sensitization	P Value
Krameria...	15/25 = 60%	6/15 = 40%	>.3
NDMA.....	79%	12/15 = 80%	

The results of this experiment while clearly without statistical significance do support the idea that NDMA can interfere with sensitization to less potent allergens. It seems that potent allergens other than DNCB can interfere, but if weaker than DNCB will do so to a lesser degree.

Simultaneous Application of Two Weaker Sensitizers

In the previous experiments only very strong sensitizers were used as interfering allergens, and the question arose whether or not weaker allergens had this effect. The strongest sensitizers, DNCB and NDMA, sensitize the majority of subjects after a single application of a suitably strong concentration. In contrast moderately strong sensitizers, such as MEH, formalin, 1-hydrazinophthalazine (1), sensitize 2 to 25 per cent of subjects when a strong concentration is applied once to freshly irritated skin, whereas weak allergens, viz, penicillin, sulfathiazole, neomycin, nitrofurazone (Furacin), sensitize less than 2 per cent under these conditions of exposure. These criteria are arbitrary.

Monobenzyl ether of hydroquinone and 1-hydrazinophthalazine were the moderately strong allergens selected to test the possibility of interference. Of these MEH is the stronger sensitizer. About 0.5 gm of a 10% concentration in cold cream was applied to normal skin under an occlusive dressing for 2 days. This procedure was repeated 3 times within 12 to 15 days and the subjects tested for sensitization in 30 days by another application of the 10% concentration in cold cream. The frequencies of sensitization were determined for each compound independently. All these subjects were healthy white males; generally white persons are more susceptible to contact sensitization than negroes. The results were as follows:

	Expected Incidence of Sensitization (alone)	Actual Incidence of Sensitization (together)
MEH.....	6/20 = 30%	15/45 = 33%
1-hydrazinophthalazine.....	4/45 = 9%	4/45 = 9%

No interference occurred. Apparently very strong sensitizers are required for interference. As a matter of fact it has been our experience over a period of years that no interference is present when groups of 3 or 4 moderately strong and weak allergens are simultaneously applied.

COMMENT

This work is preliminary and interpretations therefore tentative. Only potent contact sensitizers can exert interference. Among these there may be a kind of heirarchy in which stronger ones can interfere with weaker ones but not vice versa. The interfering allergen must be used at concentrations which give maximal rates of sensitization while the allergenic strength of the agent to be blocked must be considerably weaker. Interference therefore is manifested only under quite special circumstances. It is purely a laboratory phenomenon. In general terms interference seems to be an instance of competitive inhibition. The stronger allergen pre-empts the antibody synthesizing mechanism so that the claims of the weaker one cannot be satisfied.

Other biological examples that might help in understanding the present phenomenon are (1) the interference phenomenon in virology: cell invasion by one virus may prevent invasion by a second, competing virus (2); (2) reticuloendothelial blockade: substances like India ink when injected in large doses can temporarily block the formation of circulating antibodies (3a); (3) vitiation of the reticulo-endothelial system by physical, chemical or surgical means: viz., x-rays and poisons prevent or delay circulating antibody production (3b); (4) immune paralysis: a massive dose of antigen can inhibit specific antibody production to itself (3c); (5) the "crowding out" or "competition of antigens" effect in classic immunology: occasionally, under special circumstances the injection of one antigen prevents the formation of antibodies to another unrelated one (3d, 4). Of these examples the "crowding out" effect bears the closest resemblance to the interference phenomenon. Here too the antigenic

stimulus in terms of dose must be great but potency differences are not so clear cut (5, 6). Interestingly enough a smaller dose is satisfactory when it is given as a secondary or booster injection (7, 8). In short, the "crowding out" is evidently due to intense antibody synthesis. However, since the site of formation of antibodies in allergic contact dermatitis is not known for sure, the analogy can not be pressed too far.

SUMMARY AND CONCLUSION

(1) The interference phenomenon is the blocking of sensitization to the contactant by another unrelated one.

(2) Only the most potent contact sensitizers are capable of interference and only when applied in strong concentrations. Weaker allergens cannot block sensitization to more potent ones.

(3) The blocking effect lasts about two weeks after application of the interfering allergen.

REFERENCES

1. Mayer, R. L., Eisman, P. C. and Jaconia, D.: Experimental sensitization of guinea pigs to 1-hydrazinophthalazine; with a discussion on the use of guinea pigs for the forecast of clinical sensitizations. J. Invest. Dermat., **24:** 281, 1955.
2. Blank, H. and Rake, G.: Viral and Rickettsial Diseases of the Skin, Eye and Mucous Membranes of Man, pg. 7. Boston, Little, Brown and Co., 1955.
3. Wilson, G. S. and Miles, A. A.: Principles of Bacteriology & Immunity, Volume II: a) pg. 1255; b) pg. 1254; c) pg. 1262; d) pg. 1263. Baltimore, Williams & Wilkins Co., 1955.
4. Edsall, G.: The nature of the antigen and the basic characteristics of the immune response. J. Allergy, **28:** 1, 1957.
5. Glenny, A. I. and Waddington, H.: The immunity index method of testing antigenic values. J. Path. & Bact., **31:** 466, 1928.
6. Doerr, R. and Berger, W.: Gloubulin und albumin: aus deselben blutserum als immunisatorische antognisten. Biochem. Ztschr., **131:** 13, 1922.
7. Barr, M. and Llewellyn-Jones, M.: Some factors influencing the response to immunization with single and combined prophylactics. Brit. J. Exper. Path., **36:** 147, 1955.
8. Adler, F. L.: Antibody formation after injection of heterologous immune globulin. 2. Competition of antigens. J. Immunol., **78:** 291, 1957.

DISCUSSION

Dr. Herman N. Eisen (St. Louis, Mo.): In the past few years a number of people have begun to wonder about the possibility that perhaps a single cell is incapable of giving an allergic or immunologic response to more than one antigenic substance. To this end, Dr. William T. Newton and I have done some experiments which are quite different from those described by Dr. Epstein and Dr. Kligman, but which have some implications for their work.

When one injects into guinea pig footpads dinitrophenylprotein conjugates, the animals develop anaphylactic sensitivity specific for the dinitrophenyl group; they do *not* develop delayed skin sensitivity of the contact type to the dinitrophenyl group. Following footpad injections of dinitrophenyl-conjugated protein, we injected into the same footpads a simple dinitrophenyl sensitizer in an amount which ordinarily produces contact skin sensitization (specific for the dinitrophenyl group) in about half the animals. We were quite pleased to see that under these circumstances no contact skin sensitization was produced, even if the simple sensitizer was injected only one hour after the sensitizer-protein conjugate.

In trying to analyze the situation further, it turned out that the interference we observed was not immunologically specific. Since we routinely make our sensitizing injections with sensitizer or sensitizer-protein conjugates incorporated in Freund's adjuvant, we repeated the above experiments by making the primary injection with Freund's adjuvant alone, omitting the dinitrophenyl-protein conjugate; again a second injection into the same site with the simple dinitrophenyl sensitizer failed to induce contact skin sensitivity. Even when the primary injection consisted of only incomplete Freund's adjuvant (i.e. mineral oil, Arlacel, saline; no mycobacteria), the second injection of simple sensitizer failed to induce contact skin sensitivity. Consequently the interference we observed is not specific in any immunologic sense.

The first slide which Dr. Epstein and Dr. Kligman showed is a beautiful dose-response curve, except for the dip at the very high concentrations. Is this a real drop? It is possible I sup-

pose that the physiological effects of putting strongly irritant solutions on the skin will reduce the inducing potency of the sensitizer in a manner analogous to what Newton and I have observed in guinea pig footpad injections. If this is the case, the interference may not be on the immunologic basis envisioned by Dr. Epstein and Dr. Kligman.

Dr. Marion B. Sulzberger (New York, N. Y.): These are fascinating studies and I congratulate Bill Epstein and Kligman on pursuing so carefully and with such interest this field of investigation. At times one may feel that this is such an old field, that it has been tilled so much, that it is perhaps no longer fertile; but investigations of this type show how many new problems can arise and how many new approaches to old problems are still possible here. As I have said often, I think this is still one of the most fascinating and one of the most fruitful avenues for study in dermatology and in both applied and theoretical immunology. I am particularly glad to see that Dr. Epstein and his co-worker carried out this basic work directly in man and not in laboratory animals. Research dermatologists are fortunate in being able to exclude a great many variables and inexplicable, sometimes diametric differences of species responses by carrying out their studies directly on the living tissues of man, on the object whose immunologic reactions are of the most, if not exclusive interest to us as physicians.

I would like to ask Dr. Epstein something about the concentrations they used. I am too ignorant to be able to translate the molar concentrations he mentioned into percentages. You will perhaps recall the experiments that Dr. Landsteiner, Dr. Rostenberg, Jr., and I carried out with these same two allergens about 20 years ago. (J. Invest. Derm. **2:** 25 (Feb.) 1939). To explain them briefly,—we applied a minim of a solution of each of these allergens, a single drop from a tuberculin syringe similar to the manner which the present authors have done, but simultaneously to each of the forearms of our human test subjects. The solutions we used were a 10 per cent concentration of dinitrochlorobenzene and a 10 per cent concentration of para-nitrosodimethyl aniline. By observing both the spontaneous flare-up reactions and the reactions to subsequent challenging skin tests with higher non-irritating dilutions, we were able to demonstrate that these individuals became sensitized in a selective manner. Some became about eqally sensitive to both of the chemicals; some became sensitive to neither; some more strongly sensitive to para-nitrosodimethyl aniline than to dinitrochlorobenzene; and some vice versa. In other words the individuals were selective as regards the degree of sensitivity they developed to each compound and also to some measure as to whether or not they would become sensitized at all. We felt that this *selectivity* itself could quite obviously have nothing to do with their psychic state or their other dietary or nutritional state, hormonal state, etc., because the solutions were dropped at the same instant one on the left and one on the right on symmetrically situated areas of the forearms and the subjects had no knowledge of the nature of the chemicals or the purposes of the experiment. While I think it a fair assumption that both arms of a given subject would generally be about equal, in their "psychic state" and immunologic lability, we even ruled out the possibility of constant right and left differences by switching arms, i.e. dropping one chemical sometimes on the right arm and the same one sometimes on the left.

In the light of our results I would like to know what Dr. Epstein's concentrations were compared to the 10 per cent acetone dilution that we used. Of course the concentration he employed has very direct bearing on the problem which has been mentioned by the two previous discussers. Around a 10 per cent concentration in acetone, dinitrochlorobenzene and para-nitrosodimethyl aniline both become primary irritants. So I would like to ask the presenters in what way they can be certain that the interference phenomenon which they have demonstrated here (not only to their own satisfaction but I think also to ours), how they can be certain that this inhibitory interference is the result of any immunological action at all and is not the result of having irritated the skin with the primary irritant. The crucial experiment would to my mind be one to ascertain what happens when one attempts to sensitize with a "weak allergen" after a preceding primary irritation of the skin by something which is not a sensitizer. I assume that this has not yet been done. But I am sure Dr. Epstein will include this sort of control in these im-

portant studies, which I hope he is planning to continue.

DR. WILLIAM L. EPSTEIN (in closing): I would like to thank the discussants for their comments. It would appear they have all come to the same point. It is true that as you increase the concentrations of the allergens you get a fall off in sensitizing capacity of these compounds and particularly the fall off with para-nitrosodimethyl aniline seems significant; it runs as low as 50 per cent with the one molar concentration which is very irritating. Unfortunately this has not withstood statistical analysis because we have not yet enough subjects; but there is evidence in other areas that irritation will reduce the frequency of sensitization. There is no question about the local effect of irritants. We, Dr. Rockwell (J.I.D. **24:** 35, 1955) and many others, have shown that severe local irritation will depress the frequency of sensitization to allergens applied at that site. I must remind you I tried to point out several times that we applied these compounds at *different* non-irritated sites. I think the subtle changes that might occur at a far distant site because of irritation from the more potent allergen are minimal under these circumstances and so this is not an important criticism of the interference phenomenon. (Following this discussion further control subjects were tested with 1 molar NDMA and the results added to the original figures. The final figure, 32/45 = 71%, occurs in the first figure of the paper. This suggests that the original findings (16/27 = 59%) were the result of testing too small a sample.)

Concerning the concentrations which Dr. Sulzberger asked about; for dinitrochlorobenzene the one molar concentration is approximately 20 per cent. We used 0.5 molar, 10 per cent, which is the same as the concentrations used by Dr. Sulzberger and co-workers (J. Immun. **36:** 17, 1939), but the total amount of material applied by us was much greater than his (0.25 ml vs. 0.03 ml).

As far as Dr. Eisen's findings that the injection of Freund's adjuvant decreased sensitization to secondarily injected dinitrochlorobenzene, it is possible this was strictly an irritant effect, but it is also possible there has been a change in the reticuloendothelial system as a result of the adjuvant, which prevents the R.E.S. from taking up enough DNCB-protein for sensitization. There is fairly good evidence now that certain substances, including P.V.P. (polyvinyl pyrollidone) alter the reticuloendothelial system so that it does not take up labeled colloids in the expected manner (Weikel & Lusky, J. Pharm. & Exptl. Therap. **118:** 148, 1956).